High-throughput epitope identification for snakebite antivenom

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Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):
High-throughput epitope identification for snakebite antivenom

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Introduction

Insight into the epitopic recognition pattern for polyclonal antivenoms is a strong tool for accurate prediction of antivenom cross-reactivity and provides a basis for design of novel antivenoms. In this work, a high-throughput approach was applied to characterize linear epitopes in 966 individual snake venom components. To identify epitopes the observed peptide specific signal intensities were mapped back into 15-mer peptides and subsequently mapped to crystal structures or homology models. As examples, P-I metalloproteinase and lys49-phospholipase A₅ from Bothrops asper (venom used in antivenom production) are presented here.

Objectives

- Identify epitopes in toxins used in immunization
- Characterize tolerated amino acid substitutions in identified epitopes
- Predict cross-reactivity of antivenom

Epitopes locate to surface regions

To identify epitopes the observed peptide specific signal intensities were mapped back into 15-mer peptides with median signals above 20 AU. In this study, a high-throughput approach was applied to characterize linear epitopes in 966 individual snake venoms from pit vipers (Crotalidae) using the ICP Crotalidae antivenom. Due to an abundance of snake venom metalloproteinases and phospholipase A₅ in the venoms used for production of the investigated antivenom, this study focuses on these toxin families.

Studying linear epitopes using peptide microarrays

In silico generation of peptide library

Antibody binding and detection

Synthesis on microarray

Data analysis and protein modeling

Effect on cross-recognition

The epitope core sequences are highlighted in blue except for the high-signal epitope in Bothrops asper P-I metalloproteinase that is highlighted in red. All epitopes are found to be exposed on the protein surface. The structure of the metalloproteinase (PDB: 2W13) was obtained from the Protein Data Bank (pdb.org) and the homology model of the phospholipase A₅ was built using CPHmodels based on a crystal structure of the lys49-phospholipase from B. jararaca (PDB: 4K81) with 87.7% identity.

The α-helical shape of the epitope in the B. asper metalloproteinase is found to be highly conserved among pit viper metalloproteinases. Based on multiple sequence alignment of pit viper toxins sharing at least seven of the eight 15-mer peptides harboring the epitope, we find that flanking residues outside of the core epitope has small effect on antivenom recognition. Expanding the analysis to the 42 toxins that share at least five of the epitope residues, binding is still observed in all of the corresponding eight 15-mer peptides, although the microarray signals are reduced up to seven times (data not shown).

The results suggest that ICP Crotalidae polyvalent antivenom might offer protection against all Crotalidae venoms and might offer protection against all Crotalidae venoms and might offer protection against all Crotalidae venoms and might offer protection against all Crotalidae venoms.

Conclusions

- Custom-designed high density peptide microarray technology enables parallel automated identification of epitopes in hundreds of toxins.
- Integrating multiple sequence alignment allows investigation of the effect of epitope variation on antivenom recognition.
- Cross-reactivity of antivenom is correlated to the degree of conservation in toxin epitopes and flanking residues.

Acknowledgement

The peptide microarray experiments were performed at Schafer-N, Copenhagen. We would like to thank Claas Schuler, Christian Ajkai Hansen, and Jens Kristian for experimental setup and support. We further thank the Novo Nordisk Foundation for financial support (grant number: NNF13OC000613).

References